PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
Cynthia Webb
Webb & Associates
P.O. Box 2189
Rehovot 76121
Israel

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference KIDUM/005 PCT	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/IL 05/00230	International filing date *(day/month/year)* 24 February 2005 (24.02.2005)
Applicant STATE OF ISRAEL, MINISTRY OF AGRICULTURE, AGRICULTURAL RESEARCH ORGANIZATION	

Date of mailing *(day/month/year)* 12 FEB 2009

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 8270

   For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference KIDUM/005 PCT	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/IL 05/00230	International filing date *(day/month/year)* 24 February 2005 (24.02.2005)	(Earliest) Priority Date *(day/month/year)* 26 February 2004 (26.02.2004)
Applicant STATE OF ISRAEL, MINISTRY OF AGRICULTURE, AGRICULTURAL RESEARCH ORGANIZATION		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 5

☐ as suggested by the applicant.

☒ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 05/00230

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of:

   a. type of material
   - [x] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material
   - [ ] on paper
   - [x] in electronic form

   c. time of filing/furnishing
   - [ ] contained in the international application as filed
   - [x] filed together with the international application in electronic form
   - [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 05/00230

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to from a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-33 are directed to either an isolated enzyme, an isolated polynucleotide encoding an enzyme, a genetically modified cell, or a transgenic organism, where spacer sequence SEQ ID NO 1 will be searched without an additional search fee. Applicant may have additional sequence(s) searched upon paying additional search fee(s).

Group II: Claims 34-71 are directed to either a method for treating a disease, a method for mediating site-specific excision, or a a method for mediating site-specific insertion.

----------Continued on Extra Sheet----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-33 limited to SEQ ID NO 1.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 05/00230

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 9/22 (2009.01)
USPC - 435/199
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8)- C12N 9/22 (2009.01)
USPC- 435/199, 193, 196, 197, 252.3, 320.1, 325, 462, 463; 800/3, 13, 18, 21, 288; 536/23.1, 23.2, 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); Google Patents; Google Scholar
polyA, vetor, promoter, circular dna, genomic dna, inversion, excision, insertion, translocation, site-specific, asymmetric, recombina$, Cre, FLP, wild-type, acgtatgc, untranslated region

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/0003435 A1 (BASZCZYNSKI et al.) 01 January 2004 (01.01.2004) (para [0013]-[0015], [0019], [0025], [0031], [0032], [0038], [0042]-[0044], [0085], [0130],	1-4, 6, 7, 9-17, 20-33
Y		5, 8, 18, 19
Y	LEE et al. Role of nucleotide sequences of loxP spacer region in Cre-mediated recombination Gene 216 (1998) 55?65 (pg 59 Fig. 3 No. 21)	5, 8, 19
Y	SANTORO et al. Directed evolution of the site specificity of Cre recombinase PNAS April 2, 2002 vol. 99 no. 7 4185?4190 (pg 4185 para 4; pg 4187 Fig. 3, pg 4188 library screening)	18, 19

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 January 2009 (22.01.2009)	12 FEB 2009

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL 05/00230

Continuation of Box No. III. Lack of Unity:

The inventions of the listed groups do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature that links Group I and Group II is an enzyme capable of mediating a site-specific recombination between two predetermined recombination sites, wherein at least one recombination site is an asymmetric recombination site.. However, this is not an improvement over the prior art article entitled 'Sequence-specific and Non-specific Binding of the Rci Protein to the Asymmetric Recombination Sites of the R64 Shufflon' (Gyohda et al. Journal of Molecular Biology Volume 318, Issue 4, 10 May 2002, Pages 975-983) which teaches an enzyme capable of mediating a site-specific recombination between two predetermined recombination sites, wherein at least one recombination site is an asymmetric recombination site (pg 980, Fig 1; pg 981, col 1; and the abstract).

Accordingly, unity of invention is lacking under PCT Rule 13.2 because the groups do not share a same or corresponding special technical feature providing a contribution over the prior art.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
Cynthia Webb
Webb & Associates
P.O. Box 2189
Rehovot 76121
Israel

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	12 FEB 2009
Applicant's or agent's file reference KIDUM/005 PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL 05/00230	24 February 2005 (24.02.2005)	26 February 2004 (26.02.2004)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12N 9/22 (2009.01)
USPC - 435/199

Applicant STATE OF ISRAEL, MINISTRY OF AGRICULTURE, AGRICULTURAL RESEARCH ORGANIZATION

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	22 January 2009 (22.01.2009)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IL 05/00230

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:

   a. type of material

      ☒ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ on paper

      ☒ in electronic form

   c. time of filing/furnishing

      ☐ contained in the international application as filed

      ☒ filed together with the international application in electronic form

      ☐ furnished subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IL 05/00230

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to from a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-33 are directed to either an isolated enzyme, an isolated polynucleotide encoding an enzyme, a genetically modified cell, or a transgenic organism, where spacer sequence SEQ ID NO 1 will be searched without an additional search fee. Applicant may have additional sequence(s) searched upon paying additional search fee(s).

Group II: Claims 34-71 are directed to either a method for treating a disease, a method for mediating site-specific excision, or a a method for mediating site-specific insertion.

The inventions the listed groups do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature that links Group I and Group II is an enzyme capable of mediating a site-specific recombination between two predetermined recombination sites, wherein at least one recombination site is an asymmetric recombination site.. However, this is not an improvement over the prior art article entitled 'Sequence-specific and Non-specific Binding of the Rci Protein to the Asymmetric Recombination Sites of the R64 Shufflon' (Gyohda et al. Journal of Molecular Biology Volume 318, Issue 4, 10 May 2002, Pages 975-983) which teaches an enzyme capable of mediating a site-specific recombination between two predetermined recombination sites, wherein at least one recombination site is an asymmetric recombination site (pg 980, Fig 1; pg 981, col 1; and the abstract).

Accordingly, unity of invention is lacking under PCT Rule 13.2 because the groups do not share a same or corresponding special technical feature providing a contribution over the prior art.

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-33 limited to SEQ ID NO 1.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IL 05/00230

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	5, 8, 18, 19	YES
	Claims	1-4, 6,7, 9-17, and 20-33	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-33	NO
Industrial applicability (IA)	Claims	1-33	YES
	Claims	none	NO

2. Citations and explanations:

Claims 1-4, 6,7, 9-17, and 20-33 lack novelty under PCT Article 33(2) as being anticipated by US 2004/0003435 A1 to BASZCZYNSKI et al.(hereinafter "Baszczynski").

As per claim 1, Baszczynski discloses an isolated enzyme capable of mediating a site-specific recombination between two predetermined recombination sites, wherein at least one recombination site is an asymmetric recombination site (para [0130], [0032] ATTC is non-palindromic with respect to GTAT).

As per claim 2, Baszczynski discloses wherein the recombination is selected from a group consisting of: inversion of a first DNA molecule encompassed within a second DNA molecule, excision of a first DNA molecule from a second DNA molecule, insertion of a first DNA molecule into a second DNA molecule and translocation between a first DNA molecule and a second DNA molecule (para [0019]).

As per claim 3, Baszczynski discloses wherein the second DNA molecule is selected from the group consisting of: genomic DNA and circular DNA (para [0019]).

As per claim 4, Baszczynski discloses wherein the second DNA molecule is genomic DNA and the first DNA molecule is integrated into a predetermined genomic site selected from gene promoters (para [0038]).

As per claim 6, Baszczynski discloses a plurality of isolated enzymes (para [0025], [0130]) capable of mediating site-specific recombination between two predetermined recombination sites, wherein at least one of the recombination sites is an asymmetric recombination site (para [0032]).

As per claim 7, Baszczynski discloses wherein at least one enzyme is a wild type recombinase (para [0130]).

As per claim 9, Baszczynski discloses an isolated polynucleotide encoding an enzyme capable of mediating site-specific recombination (para [0085]) between two recombination sites, wherein at least one of the recombination sites is an asymmetric recombination site (para [0032]).

As per claim 10, Baszczynski discloses wherein said isolated polynucleotide is encompassed in a recombinant vector that expresses the at least one recombinase (para [0085]).

As per claim 11, Baszczynski discloses wherein the recombinant vector is a naked DNA plasmid (para [0085]).

As per claim 12, Baszczynski discloses wherein the recombinant vector further comprises a promoter (para [0042]).

As per claim 13, Baszczynski discloses wherein the promoter is derived from a plant (para [0042]- [0044]).

As per claim 14, Baszczynski discloses wherein the promoter is f)-actin promoter (para [0044]).

As per claim 15, Baszczynski discloses wherein the promoter is an inducible promoter (para [0043]).

As per claim 16, Baszczynski discloses wherein the inducible promoter is heat shock, or steroid hormone (para [0043]).

As per claim 17, Baszczynski discloses wherein said isolated polynucleotide encodes a plurality of enzymes (para [0025], [0085], [0130]), the plurality of enzymes is capable of mediating site-specific recombination between two predetermined recombination sites, wherein at least one of the recombination sites is an asymmetric recombination site (para [0032]).

----------Continued on Extra Sheet----------

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V. 2. Citations and explanations:

As per claim 20, Baszczynski discloses a host cell comprising a vector, the vector encompassing a polynucleotide encoding at least one enzyme, the at least one enzyme is capable of mediating site-specific recombination between two recombination sites, wherein at least one of the recombination sites is an asymmetric recombination site (para [0032], [0085]).

As per claim 21, Baszczynski discloses the host cell according to claim 20, capable of expressing said at least one enzyme (para [0042]).

As per claim 22, Baszczynski discloses a genetically modified cell transformed by an site-specific recombination between two recombination sites, wherein at least one of the recombination sites is an asymmetric recombination site (para [0032]), and , wherein the asymmetric recombination is insertion (para [0015]).

As per claim 23, Baszczynski discloses wherein the recombination occurs between the cellular endogenous genome and an exogenous DNA molecule (para [0014]).

As per claim 24, Baszczynski discloses wherein said genetically modified cell comprises an exogenous DNA molecule, wherein the exogenous DNA molecule is integrated by recombination between two recombination sites (para [0013]), at least one of the recombination sites is. an asymmetric recombination site (para [0032]), into a predetermined locus within the cellular genome (para [0014]).

As per claim 25, Baszczynski discloses wherein said genetically modified cell is eukaryotic (para [0013]).

As per claim 26, Baszczynski discloses wherein said genetically modified cell is a plant cell (para [0013]).

As per claim 27, Baszczynski discloses a transgenic organism comprising the genetically modified cell of claim 22 (para [0013]).

As per claim 28. Baszczynski discloses the transgenic organism according to claim 22, said transgenic organism is a plant (para [0013]).

As per claim 29, Baszczynski discloses wherein said cell is devoid of an endogenous polynucleotide sequence at a predetermined genomic locus (para [0034], the flp recombinase can be utilized for excision).

As per claim 30, Baszczynski discloses wherein said genetically modified cell is eukaryotic (para [0013]).

As per claim 31, Baszczynski discloses wherein said genetically modified cell is a plant cell (para [0013]).

As per claim 32, Baszczynski discloses a transgenic organism comprising the genetically modified cell of claim 29 (para [0013]).

As per claim 33, Baszczynski discloses wherein said transgenic organism is a plant (para [0013]).

Claims 5, and 8 lack an inventive step under PCT Article 33(3) as being obvious over Baszczynski in view of the article entitled " Role of nucleotide sequences of loxP spacer region in Cre-mediated recombination" by LEE et al. (hereinafter "Lee").

As per claim 5, Baszczynski discloses wherein said isolated enzyme is FLP or a modified FLP (para [0130]), mediating recombination between two recombination sites, such that at least one recombination site is an asymmetric recombination site comprising a spacer sequence (para [0031], [0032], [0130]).
Baszczynski does not disclose wherein at least one recombination site is an asymmetric recombination site comprising a spacer sequence consisting of: SEQ ID NO: 1.
Lee discloses the spacer sequence in the Cre recombination site consisting of SEQ ID NO: 1 (pg 59 Fig. 3 No. 21). It would have been obvious to use the spacer variant as taught by Lee, in the site-specific recombination system/method taught by Baszczynski, to obtain the invention as claimed, because it provides additional flexibility in achieving pre-determined genetic modifications of organisms.

As per claim 8, Baszczynski discloses wherein at least one enzyme is a Flp mutant (para [0130]) mediating recombination between two recombination sites, such that at least one recombination site is an asymmetric recombination site (para [0032]), but does not disclose a spacer sequence consisting of: SEQ ID NO: 1.
Lee discloses the spacer sequence in the Cre recombination site consisting of SEQ ID NO: 1 (pg 59 Fig. 3 No. 21). It would have been obvious to use the spacer variant as taught by Lee, in the site-specific recombination system/method taught by Baszczynski, to obtain the invention as claimed, because it provides additional flexibility in achieving pre-determined genetic modifications of organisms.

Claim 18 lacks an inventive step under PCT Article 33(3) as being obvious over Baszczynski in view of the article entitled " Directed evolution of the site specificity of Cre recombinase" by SANTORO et al. (hereinafter "Santoro").

As per claim 18, Baszczynski discloses the isolated polynucleotide according to claim 17, but does not disclose wherein each of the plurality of recombinases recognizes at least one half of the at least one asymmetric recombination site.
Santoro discloses Cre recombinase mutants wherein each of the plurality of recombinases recognizes at least one half of the at least one asymmetric recombination site (pg 4185 para 4; pg 4187 Fig. 3, pg 4188 library screening). It would have been obvious to use the Cre mutants as taught by Santoro, in the site-specific recombination system/method taught by Baszczynski, to obtain the invention as claimed, because it provides additional flexibility in achieving pre-determined genetic modifications of organisms.

----------Continued on Extra Sheet----------

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Second Continuation Page of Box No. V. 2. Citations and explanations:

Claim 19 lacks an inventive step under PCT Article 33(3) as being obvious over Baszczynski, in view of Santoro, and further in view of Lee.

As per claim 19, Baszczynski discloses the isolated polynucleotide according to claim 17, such that at least one recombination site is an asymmetric recombination site (para [0032]) but does not disclose wherein at least one recombinase is a Cre mutant mediating recombination between two recombination sites comprising a spacer sequence consisting of: SEQ ID NO: 1.
Santoro discloses Cre recombinase mutants wherein each of the plurality of recombinases recognizes at least one half of the at least one asymmetric recombination site (pg 4185 para 4; pg 4187 Fig. 3).
Lee discloses the spacer sequence in the Cre recombination site consisting of SEQ ID NO: 1 (pg 59 Fig. 3 No. 21). It would have been obvious to use the Cre mutants as taught by Santoro, for recombination of sites comprising the spacer taught by Lee, in the site-specific recombination system/method taught by Baszczynski, to obtain the invention as claimed, because it provides additional flexibility in achieving pre-determined genetic modifications of organisms.

Claims 1-33 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.